An Irradiated Electrolytic Iron Fortificant Is Poorly Absorbed by Humans and Is Less Responsive than $FeSO_4$ to the Enhancing Effect of Ascorbic Acid[1–3]

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Abstract

Despite extensive use, information on the bioavailability of elemental iron powders to humans, as influenced by dose and other dietary constituents, is limited. Three experiments were conducted to assess the absorption of electrolytic iron powder relative to $FeSO_4$, as affected by iron dose and by ascorbic or phytic acid. Iron absorption by 56 volunteers was measured from a farina cereal breakfast radiolabeled with $^{59}FeSO_4$ or an electrolytic ^{55}Fe powder irradiated by neutron activation. Absorption was determined from whole-body counting (^{59}Fe) and blood isotope incorporation 2 wk later. Absorption of iron from the irradiated electrolytic powder was 5–15% that of $FeSO_4$. Ascorbic acid (~160 mg) enhanced iron absorption from $FeSO_4$ by almost 4-fold but only doubled absorption from electrolytic iron (P for interaction < 0.01). Phytic acid from wheat bran inhibited iron absorption from $FeSO_4$ and electrolytic iron by 73 and 50%, respectively (P for interaction, NS). Compared with 3 mg, a 20-mg dose reduced fractional absorption from $FeSO_4$, but not electrolytic iron (P for interaction < 0.0001). Despite a much higher bioavailability (50% relative to $FeSO_4$) of this same electrolytic iron when tested previously in a pig model, the bioavailability of the irradiated electrolytic iron was poor in humans. The diminished influence of ascorbic acid on the absorption of less soluble iron sources such as elemental iron powders may be an important consideration when choosing iron fortificants. J. Nutr. 136: 2167–2174, 2006.

Introduction

Effective food fortification strategies are needed to combat the global problem of iron deficiency and its associated anemia. Although ferrous sulfate is a well-absorbed form of iron for dietary fortification or supplementation, it is associated with poor compliance in supplemental amounts, and with discoloration and reduced shelf life in fortified grain products (1). Elemental iron powders are relatively economical, without adverse organoleptic effects on fortified foods, but their usefulness for fortification of agricultural products is uncertain because few bioavailability or efficacy studies have been conducted in humans.

Elemental iron powders are generally characterized by production method as carbonyl, electrolytic, or reduced iron, and are composed of relatively pure (>98% iron; zero oxidation state) metallic iron (2). Compared with iron salts, the elemental iron powders have lower bioavailability for absorption, which is directly associated with their lower solubility and surface area (3–7). Human absorption of iron from such powders is difficult to assess because powders prepared for research using iron isotopes do not have the same physical characteristics as their commercial counterparts (8–15).

Isotopic labeling (^{55}Fe) of a commercial carbonyl iron powder by neutron irradiation yielded unexpectedly low measures of 5–20% bioavailability relative to $^{59}FeCl_3$ used to label the nonheme iron of meals (16). It was not clear whether those results indicated a low bioavailability of carbonyl iron or methodological difficulties with radiolabeling that changed the physical properties of the powder. However, radiolabeling by neutron activation yielded an electrolytic iron powder that was ~50% as bioavailable as ferrous sulfate in a pig model (17). This is similar to the bioavailability of nonirradiated electrolytic iron tested in a hemoglobin repletion model with rats (7). This similar bioavailability with 2 different models suggested that the same irradiated electrolytic powder may prove useful to evaluate the bioavailability of electrolytic iron in humans.

The objectives of this study were as follows: *1*) to determine the human absorption of a commercially produced, subsequently irradiated electrolytic elemental iron powder, relative to ferrous sulfate; and *2*) to assess how absorption of these iron sources is affected by ascorbic acid, phytic acid, and an increased iron dose.

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[3] Mention of a trademark or proprietary product does not constitute a guarantee of or warranty for the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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Subjects and Methods

General protocol. Healthy adult men and premenopausal women were recruited to participate in a series of 3 randomized, blinded radiolabeled meal experiments. The 3 experiments, each in a 2 × 2 factorial design, measured iron absorption from ferrous sulfate or a commercially produced, subsequently irradiated (neutron activated) electrolytic iron powder with and without the following: *1*) the enhancing effect of ascorbic acid from orange juice; *2*) the inhibiting effect of phytic acid from wheat bran in the presence of ascorbic acid; and *3*) an increased dose of added iron in the presence of ascorbic acid. Each experiment lasted for 29 d and included 4 different radiolabeled farina breakfasts served on d 1, 2, 15, and 16. Iron absorption/retention was determined 2 wk after the meals (d 15 and 29) using whole-body scintillation counting of ^{59}Fe and measuring erythrocyte incorporation of both ^{59}Fe and ^{55}Fe, labeling the ferrous sulfate and electrolytic iron, respectively.

Subjects. The study was approved by the University of North Dakota's Institutional Review Board and Radioactive Drug Research Committee and by the USDA's Human Studies Review and Radiological Safety Committees. All participants gave written informed consent.

The participants were recruited locally through public advertising. Women were not pregnant or breast-feeding. Participants were ineligible if they had had disorders affecting iron absorption and retention, had a hemoglobin <120 g/L, had used iron supplements in excess of 20 mg/d during the past 6 mo, were using medication (with the exception of women taking hormonal contraceptives, used for at least the previous 6 mo), had any underlying disease, or had a BMI above the 95th or below the 5th percentile. The volunteers were asked to discontinue the use of any nutritional supplements for the study duration.

Healthy adult men and women ($n = 56$) with a mean (± SEM) age of 37 ± 2 y (range: 21–65 y) and a BMI of 25.8 ± 0.5 kg/m^2 (range: 20.1–33.7) completed the study. Male and female volunteers varying in iron status were admitted because the relative bioavailability among different diets is not affected by iron status (18). The iron status (indicated by hemoglobin and serum ferritin) of subjects in each experiment is shown in **Table 1**.

Iron sources. Electrolytic iron powder (A-131, OMG Americas), was provided to us by Dr. Kurt Zinn; in 1995–6, he prepared radiolabeled samples of the electrolytic iron powder by neutron activation and used them in ^{59}Fe absorption studies with pigs (17). The iron powder was irradiated (University of Missouri Research Reactor, Columbia, MO) in vacuum-sealed quartz vials using a water bath to control the temperature at ≥37°C. The powder was held in the vacuum-sealed vials for 7–8 y, resulting in decay of ^{59}Fe and trace contaminants and leaving primarily ^{55}Fe activity (which has a half-life of 2.7 y). The irradiated electrolytic iron was used together with nonirradiated electrolytic iron from the same commercial batch to prepare the appropriate isotope and elemental iron doses for the test meals. To facilitate accurate weighing of individual doses, the electrolytic iron powders were homogenously mixed by repeated turning with powdered sugar. Uniform mixing was confirmed by measuring ^{55}Fe in multiple aliquots ($n = 8$); doses administered were further confirmed by analysis of aliquots prepared when the isotope preparation was added to the test meals.

The iron source used for comparison was ferrous sulfate monohydrate ($FeSO_4 \cdot H_2O$: Crown Technology), which was radiolabeled with $^{59}FeSO_4$ (NEN Life Sciences Products).

Labeled breakfast meals. For each 2 × 2 factorial experiment, test meals were randomized with the limitation that meals tested on consecutive days (d 1 and 2 or d 15 and 16) differed only in the iron isotope and its associated iron source. On each of these days, volunteers ate radiolabeled breakfasts at the Research Center in the morning, with fasting except for water for 8 h before and 3 h afterward. All test meals contained cooked, hot farina, prepared from 40 g dry unfortified farina, with 250 mL water followed by the addition of 120 g whole milk, 0.5 g salt, 14 g butter, and 24 g sucrose. In all cases, the fortification iron and associated isotopes were added to the farina before cooking. Additional variations are described for each experiment below.

Experiment 1 ($n = 20$) compared the absorption of iron from electrolytic iron powder and ferrous sulfate, and assessed the effects of added ascorbic acid. The farina test breakfast was fortified with 3 mg iron as either ferrous sulfate (labeled with 37 kBq $^{59}FeSO_4$, which supplied negligible (≤0.04 μg) additional iron mass) or electrolytic iron

TABLE 1 Human iron absorption from ferrous sulfate or electrolytic iron powder as affected by ascorbate, phytate, or the iron dose[1]

	−Ascorbate[2]		+Ascorbate[2]		*P*-values[3]		
	$FeSO_4$[4]	Electrolytic iron[5]	$FeSO_4$	Electrolytic iron	Fe source	Treatment	S × T
	%						
Experiment 1: $n = 20$ (7 men, 13 women); hemoglobin 149 ± 1 g/L; ferritin 39 (32, 47) μg/L							
Observed absorption	3.4^a (2.6, 4.5)	0.5^b (0.4, 0.6)	16.2^c (12.5, 21.2)	1.0^d (0.8, 1.4)	<0.0001	<0.0001	0.01
Normalized absorption[6]	5.8^a (4.7, 7.2)	0.8^b (0.6, 0.9)	27.4^c (22.1, 34.1)	1.8^d (1.4, 2.2)	<0.0001	<0.0001	0.01
	−Phytate[2,7]		+Phytate[2,7]				
Experiment 2: $n = 11$ (5 men, 6 women); hemoglobin 145 ± 4 g/L; ferritin 75 (59, 96) μg/L							
Observed absorption	9.5^a (6.8, 13.3)	0.4^b (0.3, 0.6)	2.6^c (1.9, 3.7)	0.2^d (0.1, 0.2)	<0.0001	<0.0001	NS
Normalized absorption	30.7^a (23.0, 41.0)	1.3^b (1.0, 1.8)	8.6^c (6.4, 11.4)	0.5^d (0.4, 0.7)	<0.0001	<0.0001	NS
	3 mg iron[2]		20 mg iron[2]				
Experiment 3: $n = 25$ (8 men, 17 women); hemoglobin 143 ± 3 g/L; ferritin 73 (63, 86) μg/L[1]							
Observed absorption	9.3^a (8.0, 10.8)	0.6^b (0.5, 0.7)	4.6^c (3.9, 5.3)	0.7^b (0.6, 0.8)	<0.0001	0.003	<0.0001
Normalized absorption	29.6^a (25.6, 34.1)	2.0^b (1.7, 2.2)	14.6^c (12.7, 16.8)	2.2^b (1.9, 2.5)	<0.0001	0.003	<0.0001

[1] Iron absorption and serum ferritin values are presented as geometric mean (−1 SEM, +1 SEM); hemoglobin values are means ± SEM. Blood values were sampled twice for each individual, corresponding to the test meal dates.
[2] Approximately 160 mg ascorbic acid as 180 g orange juice + 100 mg added ascorbate. This ascorbic acid was added to half the meals as a controlled variable in Expt. 1, and was included in all meals in Expts. 2 and 3.
[3] *P*-values show the main and interactive effects from the repeated-measures ANOVA. Means in a row with the same letter were not significantly different by Tukey contrasts. Significance was set at $P < 0.05$ (NS = not significant); all tests are 2-tailed.
[4] ^{59}Fe as $FeSO_4$ added to bakery-grade ferrous sulfate monohydrate.
[5] ^{55}Fe as commercially produced, subsequently irradiated electrolytic iron powder.
[6] Normalized to 23 μg/L serum ferritin (see text).
[7] Phytic acid, 611 mg added as 10 g wheat bran.

(labeled with 74 kBq ^{55}Fe from the irradiated iron, which provided ≤1.6 mg of the 3 mg total electrolytic iron). The 2 sources were tested with and without 180 g pulp-free orange juice (prepared fresh from frozen concentrate) containing ~60 mg endogenous ascorbate + 100 mg added ascorbic acid, or ~160 mg total ascorbate. Because of the low absorption of electrolytic iron powder observed in this first experiment, the same ascorbate-fortified orange juice was added to enhance absorption from all meals in the subsequent 2 experiments.

Experiment 2 ($n = 11$) compared the absorption of the 2 forms of iron (3 mg added to each meal) with and without 10 g of wheat bran added to the farina and ascorbate-fortified orange juice test breakfast. By analysis, the wheat bran added 1.6 mg iron and 611 mg phytic acid to the meal.

Experiment 3 ($n = 25$) compared the absorption of the 2 forms of iron as affected by iron doses of 3 and 20 mg added to the farina and ascorbate-fortified orange juice test breakfast.

Measurements of iron absorption. Absorption of ^{59}Fe was measured by whole-body scintillation counting (19) on d 14 and 29, expressing retention 12–14 d after each ^{59}Fe meal as a percentage of the initial whole-body activity 1–3 h after the meal. Corrections were made for background and physical decay. In a previous study (20), the slopes of semilogarithmic whole-body retention plots from 2 to 4 wk after isotope administration were not different from zero, suggesting that iron excretion was minimal and that it was not necessary to correct for endogenous excretion of iron during the 2 wk after isotope administration.

Because the whole-body counter does not detect ^{55}Fe, the ^{59}Fe absorption results obtained by whole-body counting were used with the blood concentrations of both radioisotopes to determine the absorption of ^{55}Fe (21) 13–14 d after the respective meals. Blood samples taken under fasting conditions on d 1 and 15 were used for background, and those on d 15 and 29 were used for absorption results, respectively. Blood volumes were limited to 30 mL/sample, or 90 mL total from each volunteer. Blood concentrations of ^{55}Fe were especially low because of low ^{55}Fe absorption. To optimize counting accuracy, samples prepared for scintillation counting were held and counted again after 90 d (2 half-lives of the ^{59}Fe). The final ^{55}Fe counts were at least 1.7 times background (80% were >2 times background), with excellent agreement between duplicate samples. No data were eliminated because of low counts.

The ^{55}Fe absorption results were obtained using the formula:

$$^{55}\text{Fe absorption} = {}^{55}\text{Fe blood fraction} \cdot {}^{59}\text{Fe WBC retention}/{}^{59}\text{Fe blood fraction},$$

where the isotope blood fraction was the isotope in 10 mL of blood, expressed as a fraction of the isotope administered. This formula used the average ^{59}Fe data from both ^{59}Fe meals consumed by each subject, applying the average fractional erythrocyte incorporation of absorbed ^{59}Fe for each individual. The whole-body counting method correlates well with methods using only blood incorporation (22), which, in contrast to the present study, estimate total blood volume from body weight and height (23,24), and assume 80% erythrocyte incorporation of the absorbed isotope by all subjects.

Other blood and chemical analyses. In addition to its use in radioisotopic assays, blood taken at the time of the meals (d 1 and 15) was used to measure hemoglobin (CELL-DYN 3500 System; Abbott Diagnostic Division) and serum ferritin (Immulite ferritin, Diagnostic Products, which is standardized using the WHO 2nd International Standard 80/578). C-reactive protein was measured by nephelometry (Behring Diagnostics) to help detect serum ferritin concentrations increased by inflammation. No iron absorption measures were excluded from the final analyses due to inflammation.

The iron content of the elemental iron powders mixed with sugar was determined by inductively coupled plasma-atomic emission MS (ICP/AES-MS; Optima 3100 XL; Perkin Elmer, Norwalk, CT) after digestion by dry ashing and heating with nitric acid. The iron content of the breakfasts was determined by ICP/AES-MS after digestion with concentrated nitric acid and 70% perchloric acid by method (II)A of the Analytical Methods Committee (25). Analytical accuracy for iron was 98 ± 7% using certified standard reference materials from the National Institute of Standards and Technology (NIST Standard Reference Material 1548a Typical Diet).

Phytic acid in the wheat bran was measured using the AOAC ion-exchange method, assuming 6 mol phosphorus/mol phytic acid (26).

Statistical and power analyses. Power analyses were conducted for each of the 3 experiments to determine the number of volunteers required to achieve ~90% power to detect significant ($\alpha = 0.05$) main effects and interactions, based on expectations that absorption of iron from ferrous sulfate would increase by 260% with ascorbic acid (27), would decrease by 60% with phytate as wheat bran added to a high ascorbate meal (27), and would decrease by 50% when the iron dose was increased from 3 to 20 mg/meal (28). We estimated that the influence of ascorbic acid, phytic acid, or iron dose on iron absorption would be reduced by half with electrolytic iron, compared with ferrous sulfate. Variability was estimated from our previous work (29). Volunteer recruitment allowed for 10% attrition.

Serum ferritin and iron absorption data were logarithmically transformed and geometric means are reported. Power curves were used to plot the relation between iron absorption and serum ferritin concentrations (after both variables are logarithmically transformed, power curves become linear relations). Absorption data were expressed as observed and, to facilitate comparison of results between studies, as normalized for subjects' iron status. The inverse relation between iron absorption and serum ferritin (when both variables are logarithmically transformed) was used to normalize iron absorption values (30) to a serum ferritin concentration of 23 μg/L, the iron status associated with 40% absorption of a reference dose (27), as follows:

$$\ln(\text{normalized percentage nonheme iron absorption}) = \ln(\text{percentage nonheme iron absorbed}) + \ln(\text{ferritin in } \mu\text{g/L}) - \ln(23\ \mu\text{g/L}).$$

All statistical tests were conducted using PC/SAS software [version 9.1; SAS Institute (31)]. Unless noted otherwise, comments refer to observed iron absorption values. Each of the experiments was evaluated by repeated measures ANOVA with Tukey contrasts. There were no significant effects of meal sequence. P-values < 0.05 (two-tailed) were accepted as significant. Multiple regression analyses with tests for parallelism and common intercepts were used to evaluate the relation of absorption to iron status (32).

Results

Effect of ascorbate (Expt. 1). Absorption of iron from the irradiated electrolytic iron powder was less than from ferrous sulfate ($P < 0.0001$), with and without ascorbate (Table 1). When ~160 mg ascorbic acid, an enhancer of nonheme iron absorption, was given with the breakfast meal, mean absorption of iron from the electrolytic iron powder approximately doubled, whereas iron absorption from ferrous sulfate increased nearly 4-fold ($P < 0.0001$, P for interaction < 0.01).

The subjects' iron status (serum ferritin) was inversely related to iron absorption; mathematical power curves, which become linear when both variables were logarithmically transformed (**Fig. 1***A*), were significant for 3 of the 4 meals. The differences among meals significantly affected the intercepts, but not the slopes of the 4 lines (Fig. 1*A*). Thus, compared with those with higher iron stores, subjects with low iron stores absorbed more iron and had greater differences in the absolute amounts of iron absorbed among dissimilar meals. However, consistent with the parallel slopes, the bioavailability of electrolytic iron relative to ferrous sulfate (RBV, or relative bioavailability value, which is expressed as a ratio, rather than a difference) was not affected by iron status (Fig. 1*B*). The food matrix influenced the relative bioavailability of the electrolytic iron powder. With the increase in ascorbic acid in the meal, the bioavailability of electrolytic iron relative

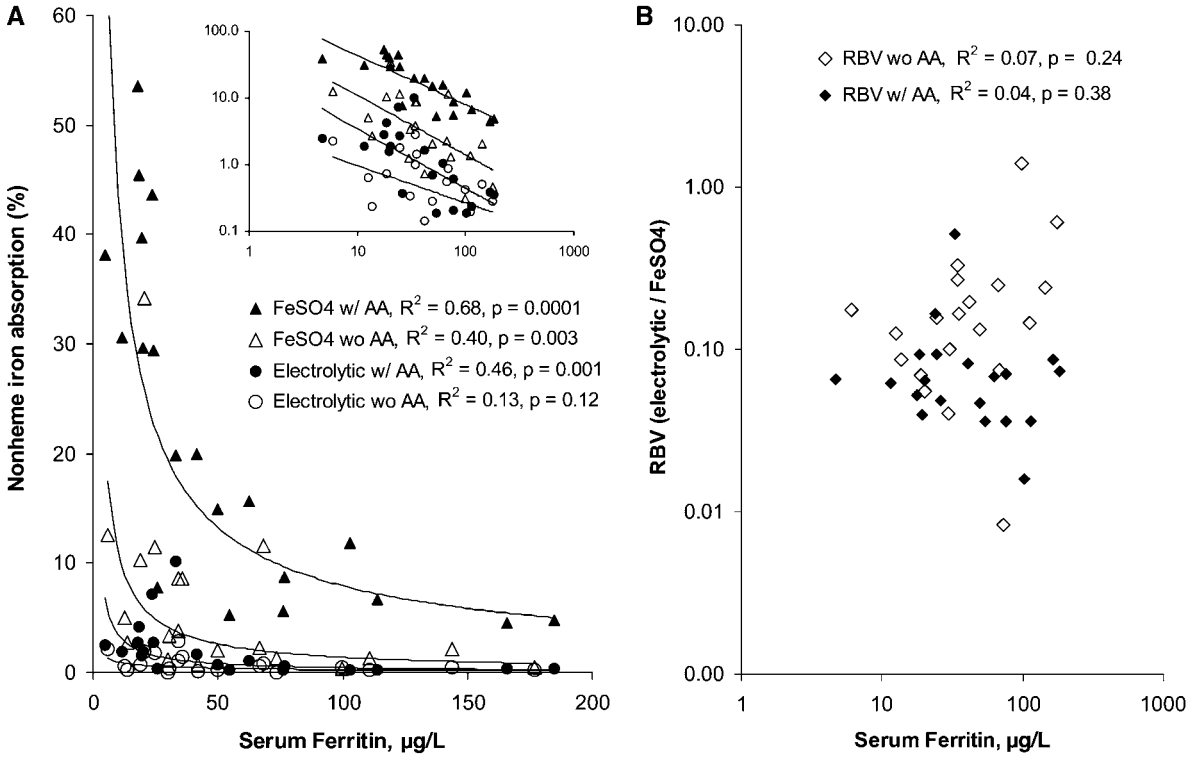

Figure 1 Human iron absorption (*A*) and RBVs (*B*) of electrolytic iron as affected by ascorbic acid (AA) (Expt. 1). (*A*) The legend for the 4 treatments is ordered from highest to lowest iron absorption. Iron absorption ($n = 20$) was inversely related to serum ferritin, significantly fitting power curves for 3 of the 4 breakfast meals. These power curves, which are linear when both axes are transformed logarithmically (see *inset* to *A*), had significantly different intercepts for the different meals, but the slopes of the lines did not differ. (*B*) Consistent with the similar slopes for absorption, the bioavailability of electrolytic iron relative to $FeSO_4$ was independent of iron status. However, the RBV of electrolytic iron was significantly greater without than with ascorbic acid (geometric means 14 vs. 6%, $P < 0.01$ by paired *t* test) because of the substantial enhancement of iron absorption from ferrous sulfate with ascorbic acid.

to ferrous sulfate decreased from 14 to 6% (geometric means; $P < 0.01$ by paired *t* test of log-transformed data) because of the greater enhancing effect of ascorbic acid on the ferrous sulfate–, compared with the electrolytic iron–fortified meal (Fig. 1*A*).

Effect of phytate (Expt. 2). When wheat bran containing phytic acid was added to the breakfast, iron absorption from ferrous sulfate and the electrolytic iron powder decreased by 73 and 50%, respectively ($P < 0.0001$; Table 1). In contrast to the main effects of the iron source (ferrous sulfate > electrolytic) and phytic acid, the interaction was nonsignificant.

Although subject numbers were more limited in Expt. 2, iron absorption again tended to vary inversely with iron stores (significant for 2 of the 4 meals), with the type of meal significantly affecting the intercepts, but not the slopes of the lines (**Fig. 2***A*). However, the bioavailability of electrolytic iron relative to ferrous sulfate was unrelated to iron status, as tested either with or without added wheat bran, and was not significantly affected by the food matrix in Expt. 2 (Fig. 2*B*).

Effect of increasing iron dose (Expt. 3). Compared with 3 mg, a 20-mg iron dose reduced the efficiency of iron absorption from ferrous sulfate from 9.3 to 4.6% (Table 1), but did not significantly affect the poor iron absorption from the electrolytic iron powder ($P < 0.0001$ for interaction between iron source and dose; Table 1). Of course, increasing the dose of iron increased the total iron absorbed, from 0.3 to 0.9 mg from the meals fortified with ferrous sulfate, and from 0.02 to 0.14 mg from the meals fortified with electrolytic iron.

As in the other experiments, iron status was inversely related to iron absorption, and differences in the iron dose significantly affected the intercept, but not the slope of the lines in the regression model (**Fig. 3***A*). Although it was not affected by the iron status of the subjects (Fig. 3*B*), the relative bioavailability of electrolytic iron was significantly greater (15 vs. 7%, $P < 0.0001$, by paired *t* test of log-transformed data) when tested with a 20-mg, rather than a 3-mg fortification dose, respectively. A dose-related change in the percentage iron absorption from ferrous sulfate, but not from electrolytic iron, accounted for this difference in relative bioavailability (Table 1) and likely reflected a partial saturation of intestinal absorption by the increased dose of the high-bioavailability ferrous sulfate.

Normalization of iron absorption for subjects' iron status. Observed iron absorption was normalized to express dietary bioavailability independently of subjects' iron stores and facilitate comparisons between these and other studies. Observed iron absorption values were normalized to a serum ferritin concentration of 23 μg/L, the iron status that is associated with 40% absorption of a reference dose (27) (see statistical methods). As anticipated, normalization of the iron absorption data yielded similar results for identical meals in experiments with different groups of volunteers. For meals with added ascorbate, no phytate, and 3 mg of iron as ferrous sulfate (Table 1), the observed values of 16.2, 9.5, and 9.3% normalized to 27.4, 30.7, and 29.6% iron absorption, in the 3 experiments, respectively. For similar meals with electrolytic iron powder, the values were 1.0, 0.4, and 0.6%, normalized to 1.8, 1.3, and 2.0% iron absorption, respectively. These similarities corroborate the

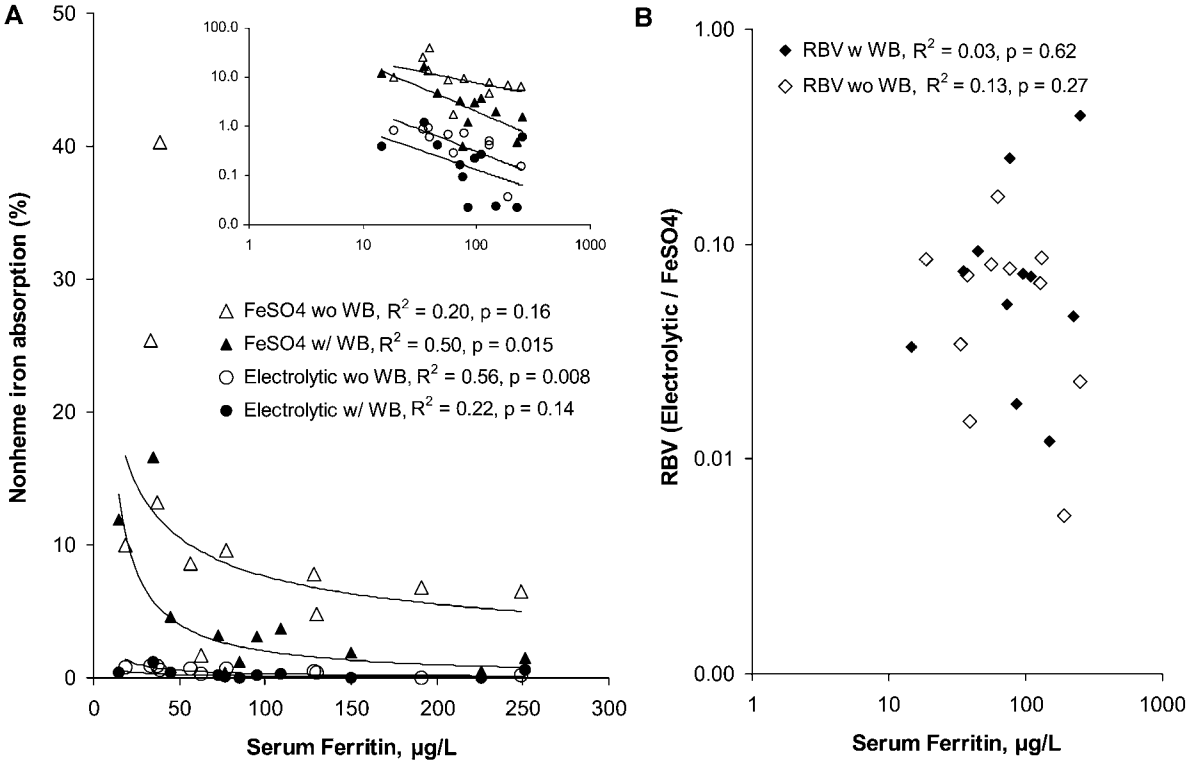

Figure 2 Human iron absorption (*A*) and RBVs (*B*) of electrolytic iron as affected by phytic acid from wheat bran (WB) (Expt. 2). (*A*) The legend for the 4 treatments is ordered from highest to lowest iron absorption. As in Figure 1, iron absorption ($n = 11$) was inversely related to serum ferritin, significantly fitting power curves for 2 of the 4 breakfast meals. The power curves are linear when both axes are transformed logarithmically (see *inset* to *A*) with significant differences in intercepts, but not slopes of the lines. (*B*) Consistent with the similar slopes in (*A*), the bioavailability of electrolytic iron relative to $FeSO_4$ was independent of iron status. The RBV of electrolytic iron was not significantly affected by wheat bran in the meal (geometric means 6% with, and 5% without wheat bran, NS by paired *t* test).

validity of the normalization procedure to correct absorption measurements for differences in iron stores of subjects.

Discussion

Although electrolytic iron powder has been widely used as a food fortificant (9,33,34), research addressing the bioavailability of this and other elemental iron powders has been limited, unfortunately, by difficulties in producing isotopically labeled powder with physicochemical properties identical to their commercially produced counterparts (9). The electrolytic iron used in the present study, irradiated under vacuum seal and at lower temperatures than the low-bioavailability carbonyl iron irradiated by Hallberg et al. (16), seemed promising for human testing because its 50% bioavailability (relative to ferrous sulfate) in growing pigs (17) was consistent with a 54% relative bioavailability of the nonirradiated commercial product as determined by hemoglobin repletion in growing rats (7). Those results were also consistent with a relative bioavailability of 50–80% for an experimentally produced electrolytic iron tested in rats and humans (9), 65% tested with plasma appearance curves in humans (24), and ~77% (35) in a human study that compared the efficacy of iron sources for increasing body iron. However, when tested in humans in the present report, the irradiated electrolytic iron was only 5–15% as bioavailable as ferrous sulfate (Figs. 1–3).

Although not consistent with results using other bioavailability methods, this low bioavailability of the irradiated electrolytic iron is consistent with the low bioavailability observed with human testing of another irradiated powder, carbonyl iron (5–20% relative to extrinsically labeled nonheme food iron) (16). Although the reason for this low iron bioavailability cannot be determined from the present data, we favor the possibility that the irradiation process altered the physical form and bioavailability of the iron powder. The limited quantity of irradiated electrolytic iron powder in our possession was insufficient to conduct physicochemical measurements. However, such measurements may not have revealed the problem. For instance, dissolution rates, which correlate well with the bioavailability of elemental iron powders for hemoglobin repletion of anemic rats (7), were similar between the irradiated and nonirradiated carbonyl iron samples tested by Hallberg et al. (16). Although possible physical changes resulting from the irradiation of carbonyl iron were discussed (2) as an explanation for the low bioavailability observed by Hallberg et al. (16), this has not been fully resolved. However, subsequent testing suggested much higher RBVs for (nonirradiated) carbonyl iron: 58% based on plasma iron appearance curves when added to wheat rolls (36) and 70% when used as a supplement to increase subjects' iron status (37). The poor bioavailability of the irradiated electrolytic iron in the present study was not likely caused by oxidation or other chemical changes during the ~7–8 y of storage because these relatively pure elemental iron samples were vacuum sealed until use. However, we cannot completely rule out a possible influence of several years of storage for radioactive decay when comparing the present study with the pig study of Zinn et al. (17). If irradiation is not a useful means of labeling elemental iron powders to measure human absorption, as the present low RBV values and those of Hallberg et al. (16) suggest, a combination of indirect or less sensitive methods must be used, including animal studies, small-scale isotopic simulation of

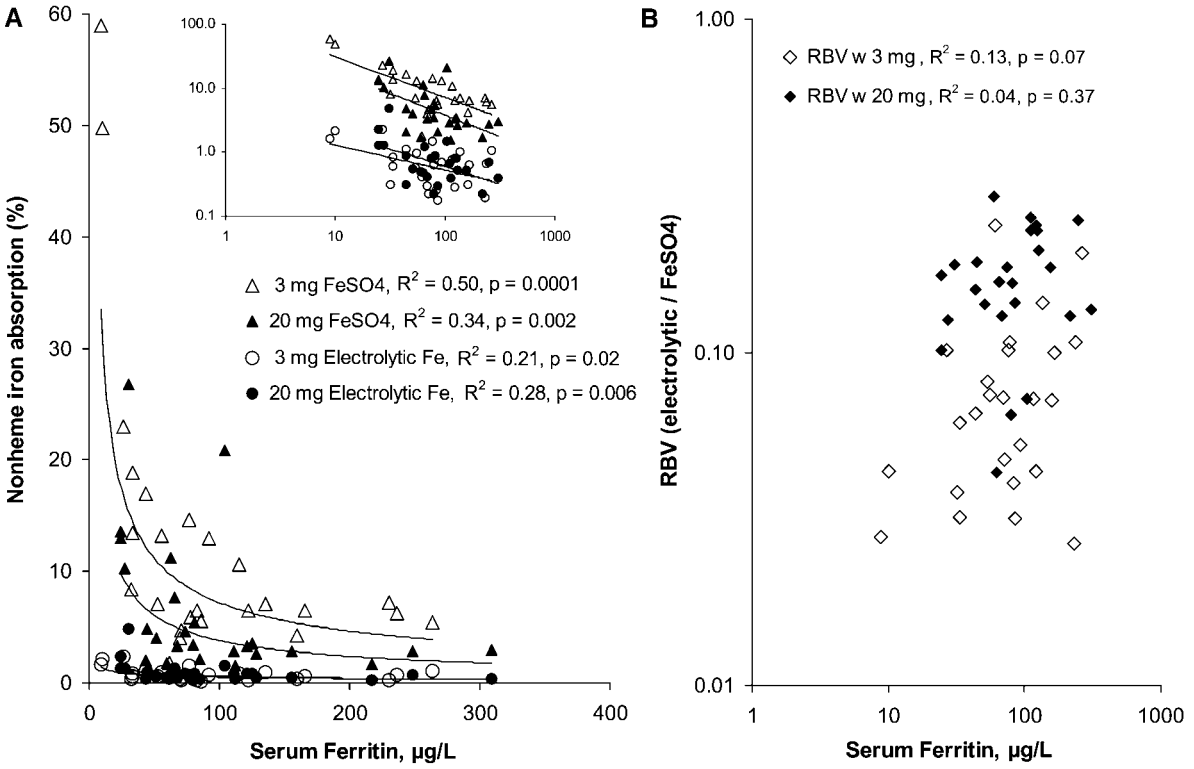

Figure 3 Human iron absorption (*A*) and RBVs (*B*) of electrolytic iron as affected by iron doses of 3 or 20 mg (Expt. 2). (*A*) The legend for the 4 treatments is ordered from highest to lowest iron absorption. As in Figure 1, iron absorption ($n = 25$) was inversely related to serum ferritin, significantly fitting power curves for each meal. The power curves are linear when both axes are transformed logarithmically (see *inset* to *A*) with significant differences in intercepts, but not slopes of the lines. (*B*) Consistent with the similar slopes in (*A*), the bioavailability of iron relative to $FeSO_4$ was independent of iron status. However, the RBV of electrolytic iron was significantly lower when tested with the lower dose of iron (geometric means of 7 vs. 15%, $P < 0.0001$ by paired *t* test) because of the more substantial influence of dose on iron absorption from ferrous sulfate.

commercial powders, plasma appearance curves, and ultimately, randomized controlled studies of changes in iron status.

Despite the low bioavailability of the irradiated electrolytic iron powder, the present results provide information on the influence of dietary modifiers of absorption, which may be applicable with less soluble forms of iron fortificants, such as the "reduced" iron powders (7,36) or salts such as ferric pyrophosphate. The results suggest that modifiers of iron absorption, such as ascorbic acid and an increased iron dose, can have a smaller effect on the absorption of a poorly available, less soluble iron source, compared with a readily ionized and soluble form of iron such as ferrous sulfate. Specifically, the 4-fold greater absorption of ferrous sulfate with the addition of ~160 mg ascorbic acid was significantly more than the 100% greater absorption of the iron powder, and compared with 3 mg, the 20-mg dose reduced iron absorption from ferrous sulfate, but not from the iron powder (Table 1). Consistent with the diminished enhancing effect of ascorbic acid on the absorption of electrolytic elemental iron of this study, a lower amount (15 mg) of ascorbic acid did not increase the absorption of a laboratory-prepared H-reduced elemental iron (13). Although Hallberg et al. (16) found the that the RBV of irradiated carbonyl iron was unaffected by the addition of 25 or 50 mg ascorbic acid, they observed differences in RBV with different meal combinations, possibly caused by differences in gastric pH and gastric emptying time that could influence dissolution of the iron powder. Moretti et al. (38) found that the RBV of a micronized, dispersible ferric pyrophosphate fortificant also depended on the food matrix; consistent with the present study, the enhancing effect of ascorbic acid was less with the fortificant (ferric pyrophosphate) than with a ferrous sulfate control. The present findings indicate the importance of considering dietary factors and the dissimilar effects they may have on different forms of iron and, consequently, on measurements of relative iron bioavailability.

Although the efficiency of iron absorption was inversely related to iron status, the present investigation did not detect any relation between relative iron bioavailability (electrolytic/ferrous sulfate) and body iron status (Figs. 1–3). This is consistent with other reports that relative bioavailability of iron from different food combinations is independent of body iron stores (18,20,39,40), including a study of 870 rural Venezuelan subjects in which one-third had low serum ferritin (<12 μg/L) (39). Moretti et al. (38) recently reported that iron status affected the relative bioavailability of ferric pyrophosphate, and that this phenomenon may extend to other poorly soluble iron compounds. The observation of Moretti and colleagues is somewhat difficult to evaluate because data from 2 studies with different meals and substantial differences in relative iron bioavailability were combined for a single correlation. Although only a few of the subjects in the present investigation had low serum ferritin, these data do not suggest any relation between iron status and another poorly soluble form, electrolytic iron. In the single instance in which such a relation approached significance ($P = 0.07$ for serum ferritin vs. RBV with 3 mg iron dose, Fig. 3*B*), identical meals with different subjects in the first 2 experiments (RBV without ascorbic acid in Fig. 1*B* and RBV without bran in Fig. 2*B*) did not confirm any relation.

In conclusion, the low bioavailability of irradiated electrolytic iron suggests that irradiation is not a useful means of

radiolabeling commercial iron powders, which also helps explain the low bioavailability observed in a previous similar study of irradiated carbonyl iron. Dietary factors that modify iron absorption, especially ascorbic acid and the iron dose administered, had a smaller effect on the absorption of this poorly bioavailable iron powder than on the absorption of ferrous sulfate. Elemental iron powders, although useful for avoiding oxidative reactions with adverse organoleptic effects in staple foods, are not only less bioavailable than more soluble ferrous iron salts, but their absorption is less likely to be enhanced by dietary modifiers such as ascorbic acid. These factors should be considered in designing food fortification programs to address national and international iron deficiency.

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Literature Cited

1. SUSTAIN: Sharing United States Technology to Aid in the Improvement of Nutrition. Guidelines for iron fortification of cereal food staples. 2001 [cited 2006 Jan 20]; Available from: http://www.micronutrient.org/resources/publications/fe_guide.pdf#search='Guidelines%20for%20Iron%20Fortification%20of%20Cereal%20Food%20Staples%20SUSTAIN'
2. Hurrell R, Bothwell T, Cook JD, Dary O, Davidsson L, Fairweather-Tait S, Hallberg L, Lynch S, Rosado J, et al. The usefulness of elemental iron for cereal flour fortification: a SUSTAIN Task Force report. Sharing United States Technology to Aid in the Improvement of Nutrition. Nutr Rev. 2002;60:391–406.
3. Pennell MD, Wiens WD, Rasper J, Motzok I, Ross HU. Factors affecting the relative biological value of food grade elemental iron powders for rats and humans. J Food Sci. 1975;40:879–83.
4. Shah BG, Giroux A, Belonje B. Specifications for reduced iron as a food additive. J Agric Food Chem. 1977;25:592–4.
5. Verma RS, Motzok I, Chen SS, Rasper J, Ross HV. Effect of storage in flour and of particle size on the bioavailability of elemental iron powders for rats and humans. J Assoc Off Anal Chem. 1977;60:759–65.
6. Motzok I, Verma RS, Chen VS, Rasper J, Hancock RGV, Ross HU. Bioavailability, in vitro solubility, and physical and chemical properties of elemental iron powders. J Assoc Off Anal Chem. 1978;61:887–93.
7. Swain JH, Newman SM, Hunt JR. Bioavailability of elemental iron powders to rats is less than bakery-grade ferrous sulfate and predicted by iron solubility and particle surface area. J Nutr. 2003;133:3546–52.
8. Cook JD, Minnich V, Moore CV, Rasmussen A, Bradley WB, Finch CA. Absorption of fortification iron in bread. Am J Clin Nutr. 1973;26:861–72.
9. Forbes AL, Adams CE, Arnaud MJ, Chichester CO, Cook JD, Harrison BN, Hurrell RF, Kahn SG, Morris ER, et al. Comparison of in vitro, animal and clinical determinations of iron bioavailability: International Nutritional Anemia Consultative Group Task Force report on iron bioavailability, bioavailability of iron used in fortification and enrichment, electrolytic iron, ferric orthophosphate, ferrous sulfate. Am J Clin Nutr. 1989;49:225–38.
10. Hoglund S, Reizenstein P. Studies on iron absorption. V. Effect of gastrointestinal factors on iron absorption. Blood. 1969;34:486–504.
11. Björn-Rasmussen E, Hallberg L, Rossander L. Absorption of 'fortification' iron. Bioavailability in man of different samples of reduced Fe, and prediction of the effects of Fe fortification. Br J Nutr. 1977;37:375–88.
12. Roe MA, Fairweather-Tait SJ. High bioavailability of reduced iron added to UK flour. Lancet. 1999;353:1938–9.
13. Fairweather-Tait SJ, Wortley GM, Teucher B, Dainty J. Iron absorption from a breakfast cereal: effects of EDTA compounds and ascorbic acid. Int J Vitam Nutr Res. 2001;71:117–22.
14. Walter T, Pizarro F, Abrams SA, Boy E. Bioavailability of elemental iron powder in white wheat bread. Eur J Clin Nutr. 2004;58:555–8.
15. Walter T, Pizarro F, Boy E, Abrams SA. The poor bioavailability of elemental iron in corn masa flour is not affected by disodium EDTA. J Nutr. 2004;134:380–3.
16. Hallberg L, Brune M, Rossander L. Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour. Am J Clin Nutr. 1986;43:59–67.
17. Zinn KR, Chaudhuri TR, Mountz JM, van den Berg GJ, Gordon DT, Johanning GL. 59Fe is retained from an elemental 59Fe powder supplement without effects on 65Zinc, 47Calcium and 67Copper in young pigs. J Nutr. 1999;129:181–7.
18. Hallberg L. Perspectives on nutritional iron deficiency. Annu Rev Nutr. 2001;21:1–21.
19. Lykken GI, Ong HS, Alkhatib HA, Harris TR, Momcilovc B, Penland JG. Perquisite spin-off from twenty-two years of measuring background in the whole body counter steel room. Ann N Y Acad Sci. 2000;904:267–70.
20. Hunt JR, Roughead ZK. Nonheme iron absorption, fecal ferritin excretion, and blood indexes of iron status in women consuming controlled lactoovovegetarian diets for 8 wk. Am J Clin Nutr. 1999;69:944–52.
21. Bothwell TH, Charlton RW, Cook JD, Finch CA. Iron metabolism in man. London: Blackwell Scientific Publications; 1979.
22. Hunt JR, Roughead ZK. Adaptation of iron absorption in men consuming diets with high or low iron bioavailability. Am J Clin Nutr. 2000;71:94–102.
23. Wennesland R, Brown E, Hopper J, Hodges JL, Guttentag OE, Scott KG, Tucker IN, Bradley B. Red cell, plasma and blood volume in healthy men measured by radiochromium (Cr51) cell tagging and hematocrit: influence of age, somatotype and habits of physical activity on variance after regression of volumes to height and weight combined. J Clin Invest. 1959;38:1065–77.
24. Brown E, Hopper J, Hodges JL, Bradley B, Wennesland R, Yamauchi H. Red cell, plasma, and blood volume in healthy women measured by radiochromium cell-labeling and hematocrit. J Clin Invest. 1962;41:2182–90.
25. Analytical Methods Committee. Methods of destruction of organic matter. Analyst. 1960;85:643–56.
26. Association of Official Analytical Chemists. Phytate in foods (method 986.11). In: Helrich K, editor. AOAC Official Methods of Analysis, 15th edition. Arlington, VA; 1990.
27. Hallberg L, Hulthen L. Prediction of dietary iron absorption: an algorithm for calculating absorption and bioavailability of dietary iron (see erratum Am J Clin Nutr 2000; 72:1242). Am J Clin Nutr. 2000;71:1147–60.
28. Werner E, Roth P, Kaltwasser JP. Relationship between the dose administered and the intestinal absorption of iron from ferrous sulphate in humans. In: Saltman P, Hegenauer J, editors. The biochemistry and physiology of iron. Elsvier North Holland;1982. p. 821–3.
29. Roughead ZK, Hunt JR. Adaptation in iron absorption: iron supplementation reduces nonheme-iron but not heme-iron absorption from food. Am J Clin Nutr. 2000;72:982–9.
30. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. Am J Clin Nutr. 1991;54:717–22.
31. SAS Institute Inc. SAS/STAT User's Guide, Version 9.1. Cary, NC: SAS Institute; 1999.
32. Kleinbaum DG, Kupper LL. Applied regression analysis and other multivariable methods. North Scituate, MA: Duxbury Press; 1978.
33. Whittaker P. Iron and zinc interactions in humans. Am J Clin Nutr. 1998;68:442S–6.
34. Hurrell RF. Fortification: overcoming technical and practical barriers. J Nutr. 2002;132:806S–12.
35. Zimmermann MB, Winichagoon P, Gowachirapant S, Hess SY, Harrington M, Chavasit V, Lynch SR, Hurrell RF. Comparison of the efficacy of wheat-based snacks fortified with ferrous sulfate, electrolytic iron, or hydrogen-reduced elemental iron: randomized,

double-blind, controlled trial in Thai women. Am J Clin Nutr. 2005;82:1276–82.
36. Hoppe M, Hulthen L, L. H. The relative bioavailability in humans of elemental iron powders for use in food fortification. Eur J Nutr. 2006;45:37–44.
37. Devasthali SD, Gordeuk VR, Brittenham GM, Bravo JR, Hughes MA, Keating LJ. Bioavailability of carbonyl iron: a randomized, double-blind study. Eur J Haematol. 1991;46:272–8.
38. Moretti D, Zimmermann MB, Wegmuller R, Walczyk T, Zeder C, Hurrell RF. Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans. Am J Clin Nutr. 2006;83:632–8.
39. Taylor P, Martinez-Torres C, Leets I, Ramirez J, Garcia-Casal MN, Layrisse M. Relationships among iron absorption, percent saturation of plasma transferrin and serum ferritin concentration in humans. J Nutr. 1988;118:1110–5.
40. Bovell-Benjamin AC, Viteri FE, Allen LH. Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. Am J Clin Nutr. 2000;71:1563–9.